TRANSLATION NO. 1269

DATE: 6 Jan 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

FURTHER REPORTS ON THE DISTRIBUTION OF NITROGEN-CONTAINING MATERIALS IN THE BLOOD

Zeitschrift für klinische Medizin
(Journal of Clinical Medicine),
Vol. 50, Nos. 3 and 4, 1903,
pages 167-168, 216-234, 252.

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In my first report ([Note]: See Jaksch, Zeitschrift für klinishche Medizin, Vol. 47, 1902, page 1) on the same subject, I called to one's attention in the conclusion that first, the Schöndorff method of urea determination gave adequate results even with less than 10 g phosphoric acid, and second, the values which I presented in this series of observations may have been too low for urea and too high for amino acid nitrogen because the total urea was not converted into ammonium phosphate due to the use of an insufficient quantity of phosphoric acid.

I will treat this question in the following observations.

Note that the method is exactly the same as that which I have already fully described previously. This time, also, on the grounds presented formerly, I deemed it necessary to present each experiment individually.

The material I have worked up for this purpose includes:

I.	Kidney affections.....	15 cases with	16 investigations
II.	Liver diseases.....	2 cases with	2 investigations
III.	Anemia.....	4 cases with	4 investigations
IV.	Diabetes mellitus.....	3 cases with	8 investigations
V.	Lobular pneumonia.....	1 case with	2 investigations
VI.	Morbus Basedowii.....	1 case with	1 investigation
VII.	Erysipelas.....	1 case with	1 investigation
VIII.	Typhus abdominalis.....	4 cases with	4 investigations
IX.	Mild rheumatism of the joints.....	1 case with	6 investigations

In addition, four experiments with normal human urine with and without the addition of hippuric acid were carried out, an experiment with horse urine and an experiment in one case on mild phosphorous poisoning. I note in addition that since the conclusion of my first series of observations, a report by M. Krüger and J. Schmid ([Note]: Krüger and Schmid, Zeitschrift für physiologische Chemie (Journal of Physiological Chemistry), Volume 31, 1900-1901, page 356) has come to my attention which is also concerned with the detection of amino acid nitrogen in urine.

My observation material now follows.

V. Lobular Pneumonia

Case XXV.

Thirty-first experiment on 7-8 December.

In the following case the patient was severely comatose when brought in; traces of sugar and albumen were found in the urine; in addition there was a high fever and signs of a lung affection. A lobular pneumonia was found in the left lung on dissection and also an abscess in the same lung; bacteriological investigation pointed to the presence of diplococcus lanceolatus.

The urine contained sugar and albumen; both substances were present in small concentrations, however, so that determining the sugar quantitatively with the polarimeter gave no result.

The experiment with native acid urine was carried out first; of course, I lacked a 24 hour-quantity of urine in this case since the urine could not be accurately collected because of the short duration of observation and the severe coma. The same experiment was then carried out with fermented urine. I first carried out the experiment on unfermented urine.

The density was 1.022. The albumen nitrogen content of the urine in 100 cc, determined in the usual way, amounted to 0.0035 g in 100 cc, 0.000175 g in 5 cc; on the average 1 cc of the acid present was used: 0.9 cc in the first experiment, 1.1 cc in the second experiment.

Five cc of urine were subjected to the Kjeldahl nitrogen determination; 17.1 cc of the acid present were used. The control experiment was inaccurate.

Five cc urine contained 0.05985 g nitrogen.
One hundred cc urine contained 1.19700 g nitrogen.

From 5 cc urine, 19.2 cc phosphotungstic hydrochloric acid precipitated the total material precipitable by this reagent. The residue from 5 cc urine used, on the average, 0.75 cc of the acid present: 0.8 cc in the first experiment, 0.7 cc in the second experiment.

The residue from 5 cc urine contained 0.002625 g nitrogen.
The residue from 100 cc urine contained 0.052500 g nitrogen.

There were 0.000175 g of albumen nitrogen in 5 cc urine (see above); 50 cc + 242 cc phosphotungstic acid produced a filtrate of 225 cc which increased in volume to 230 cc after the addition of lime; 230 cc filtrate correspond to 46.487603 cc urine, 20 cc filtrate to 4.042400 cc.

Twenty cc filtrate, treated by Schöndorff's method, consumed these quantities of the sulphuric acid present each time: using

10 g phosphoric acid, 12.15 cc acid on the average were used:
12.1 cc in the first experiment, 12.2 in the second experiment;
5 g phosphoric acid, 12.2 cc acid on the average were used:
12.2 cc in the first experiment, 12.2 in the second experiment;
3 g phosphoric acid, 12.2 cc acid on the average were used:
12.2 in the first experiment, 12.2 in the second experiment.

The results were identical whether 10 or 3 g phosphoric acid were used; 12.2 cc will be taken as a basis for further calculations because this number reappeared in four analyses. The liquid remained blue. Twenty cc filtrate equal to 4.04240 cc urine contained 0.04270 g urea nitrogen.

In 5 cc urine there were 0.052815 g urea nitrogen = 0.113183 g urea.
In 100 cc urine there were 1.056303 g urea nitrogen = 2.263657 g urea.

Twenty cc of the filtrate equal to 4.04240 cc of urine were analyzed each time by the Kjeldahl method. An average of 12.75 cc of the acid present was used, 12.7 cc in the first experiment, 12.8 cc in the second experiment. Twenty cc filtrate = 4.024 cc urine contained 0.044625 g nitrogen.

Filtrate II from 5 cc urine contained 0.055196 g nitrogen.
Filtrate II from 100 cc urine contained 0.103923 g nitrogen.

Of the total nitrogen, 88.245614% existed as urea, and 95.686281% existed as urea.

Five cc urine contained 0.05985 g nitrogen; the residue + filtrate II contained 0.057821 g, a difference of 0.002029 g. Of the 0.05985 g nitrogen in 5 cc urine, the albumen contained 0.000175 g nitrogen, the purine materials, ammonia, etc. contained 0.002450 g nitrogen, the urea 0.52815 g nitrogen and the amino acids 0.002381 g nitrogen.

Of the total nitrogen of 0.05985 g in 5 cc urine, nitrogen in the residue contributed 4.385964%.

The albumen contained.....0.292397%)
The rest of the material)
precipitable by phospho-) = 4.385964%
tungstic acid contained.....4.093567%)

The nitrogen contained in the urea was.....88.245614%.
The nitrogen contained in the amino acids was....3.978279%.
The sum is 96.609857% and the missing 3.390143% indicates the experimental error.

Thirty-second experiment.

The experiment with the fermented urine which stood in an incubator for 48 hours at 40° gave the following results. It is first noted that the nitrogen content of the yeast amounted to 1.74% on the average: 1.74251% in the first experiment, 1.74656% in the second experiment, therefore agreeing with values presented on page 213.

Five cc urine were treated each time by the Kjeldahl method. On the average, 16.5 cc of the acid present were used: 16.4 cc in the first experiment, 16.6 cc in the second experiment.

Five cc urine contained 0.05775 g nitrogen.
One hundred cc urine contained 1.15500 g nitrogen.

The total substance precipitable from 5 cc urine by phosphotungstic hydrochloric acid was precipitated by 19.2 cc of this reagent.

The residue from 5 cc was digested in the usual way, using an average of 1.05 cc of the acid present: 1 cc in the first experiment, 1.1 cc in the second experiment.

The residue from 5 cc urine contained 0.003675 g nitrogen.
The residue from 100 cc urine contained 0.73500 g nitrogen.

Fifty cc urine + 242 cc phosphotungstic hydrochloric acid produced a filtrate of 210 cc which increased to a volume of 215 cc on the addition of lime; 215 cc filtrate corresponded to 43.288429 cc urine, 20 cc to 4.036132 cc urine. The filtrate was colorless.

Twenty cc filtrate, treated by Schöndorff's method, consumed these quantities of the acid present each time: using

10 g phosphoric acid, 12.05 cc of acid on the average were used:
11.9 cc in the first experiment, 12.2 cc in the second experiment;
5 g phosphoric acid, 12.05 cc of acid on the average were used:
12.1 cc in the first experiment, 12.1 cc in the second experiment;
3 g phosphoric acid, 12.00 cc of acid on the average were used:
12.0 cc in the first experiment, --- in the second experiment.

Therefore, identical results were obtained with 10, 5, 3 g phosphoric acid and the value of 12.05 cc was taken as a basis for further calculations. Twenty cc filtrate corresponding to 4.036132 cc urine, contained 0.042175 g urea nitrogen.

Five cc urine contained 0.052246 g urea nitrogen = 0.111963178 g urea.

One hundred cc urine contained 1.044936 g urea nitrogen = 2.239297848 g urea.

Twenty cc filtrate II were treated each time by the Kjeldahl method, and one experiment was lost. In the second experiment, which was not perfect, 11.6 cc of the acid present were used; 20 cc of filtrate II = 4.036132 cc urine contained 0.04060 g nitrogen.

Filtrate II from 5 cc urine contained 0.050295 g nitrogen.
Filtrate II from 100 cc urine contained 1.00590 g nitrogen.

Of the total nitrogen not precipitable by phosphotungstic acid, 90.469264% existed as urea. The actual value obtained, 103.879113% is qualified by an experimental error in the Kjeldahl determination of nitrogen in filtrate II.

Five cc of urine contained 0.05775 g nitrogen; filtrate II + the residue contained 0.055921 g, a difference of 0.001829 g. Of the 0.05775 g nitrogen in 5 cc urine, the residue contained 0.003675 g nitrogen, the urea 0.052246 g nitrogen, the amino acids a negative value of -0.002246 g nitrogen which is due to experimental error.

Of the total nitrogen of 0.05775 g in 5 cc urine
the residue contained.....6.363636%,
the urea contained.....90.469264%,
a total of 96.83290%, and the missing 3.1671% indicates the experimental error.

Although this experiment is not entirely satisfactory because of the errors made in the Kjeldahl determination of nitrogen in filtrate II, I have mentioned it here because it shows that even a minimum quantity of sugar in the urine serves to influence the Schöndorff urea determination in the sense that less urea and correspondingly more amino acid nitrogen was found. However, the error here is not in the amount of phosphoric acid available for use since the same values for the urea nitrogen were found with 3 g phosphoric acid as with 10 g phosphoric acid. I believe that in this case the anomalous result was caused by the presence of phosphotungstic acid salts in the filtrate -- the filtrate was blue -- and therefore caused by the presence of phosphotungstic acid in the filtrate as well as by the fact that the sugar formed compounds with phosphoric acid. The Schöndorff method for urea determination in diabetic urine gives consistent results only after the removal of the sugar from the urine by fermentation. This experiment also shows, as do those preceding, that the total nitrogen in fermented urine decreases

and the residue nitrogen increases for the reasons already stated. However, the experiment indicates that we have in phosphotungstic acid a sensitive reagent for detecting sugar in the urine. I am now concerned with the elaboration of a method for the detection of sugar which arises out of this experiment, and Dr. Otori of my clinic will shortly publish a complete report concerning this.

In reference to the method for urea detection in sugar-containing urine by means of Schöndorff's method, these experiments have shown:

1. Removing the phosphotungstic acid from the filtrate is not successful, even if the urine contains only traces of sugar, perhaps because of the dissolving action of the sugar on the calcium phosphotungstate. Only a very large excess of lime precipitates the calcium phosphotungstate along with the sugar. How far the Schöndorff determination of urea is influenced by sugar in the urine will be shown shortly by the experiments of Otori.

2. The sugar forms compounds with the phosphoric acid so that a part of the urea escapes conversion into ammonium phosphate during the Schöndorff method. Ten g of phosphoric acid, as prescribed by Schöndorff (see experiments 28 and 29), are insufficient for total conversion, but 20 to 30 g are sufficient.

3. Even using 20-30 g phosphoric acid (see experiment 29), the quantity of urea nitrogen seems to be too small and that of the amino acid nitrogen too large because of the presence of phosphotungstic acid in the filtrate.

4. Therefore, of the previously mentioned methods, we chose the removal of sugar from the urine by fermentation. For, although the percent composition changes by the loss of total nitrogen (see experiments 29 and 32), and an increase of residue nitrogen by the formation of ammonium salts, the absolute values obtained for the urea and amino acid nitrogen are closest to the actual proportions.

I will therefore take these values which were obtained with fermented urine as the sole basis for my considerations on the distribution of nitrogen in the urine. The high percental values and absolute numbers which I presented in experiments 23, 24 and 25, 26 and also 28 for the amino acids originate from the above mentioned errors caused by the use of the Schöndorff method for urea determination on diabetic urine.

If we examine the results obtained from this point of view, only experiments 27 and 29 are valuable for our purpose; they show that the amino acid nitrogen amounts to 3,35-3,86% of the total nitrogen; the absolute value was 0,63-0,64 g, giving an average value of 0,64 g, which amounts to 8,16 g hippuric acid. The urea was distributed from 79,30-88,35% in the nitrogen secretion. Its quantity amounted to 27,83-36,37 g; the residue nitrogen fluctuated between 3,14-13,08%. Experiment 30 is not suitable for this

purpose since decomposed urine was used. This experiment shows that the secretion of nitrogen in the form of urea plays the most important role even in diabetes. It should not be denied that in these experiments the highest values were obtained for the amino acids, and further some very high values were even obtained for the residue nitrogen -- I am omitting the unsatisfactory experiment 30. Therefore, further experiments should be carried out on the urine of diabetics in order to answer these questions completely and conclusively.

VII. Erysipelas

Case XXVI.

Thirty-fourth experiment from 22-23 January 1903.

Erysipelas. Temperature between 39.7-40.0° C. Urine quantity 460 cc, density 1.017, no albumen, acid reaction.

Five cc urine were treated each time by the Kjeldahl method; one experiment is inaccurate; 38 cc of the normal sulphuric acid present were used in the second experiment.

In 5 cc urine there were 0.13300 g nitrogen.
In 100 cc urine there were 2.6600 g nitrogen.
In 460 cc urine there were 12.236 g nitrogen.

Eighteen cc of phosphotungstic hydrochloric acid precipitated the total substance from 5 cc urine precipitable by this reagent. The residue from 5 cc urine was digested in the usual way and used on an average 2.25 cc of the acid present: 2.3 cc in the first experiment, 2.2 cc in the second experiment.

The residue from 5 cc urine contained 0.007875 g nitrogen.
The residue from 100 cc urine contained 0.157500 g nitrogen.
The residue from 460 cc urine contained 0.724500 g nitrogen.

Fifty cc urine + 180 cc phosphotungstic hydrochloric acid produced a filtrate of 195 cc which increased to a volume of 200 cc after the addition of lime; 200 cc filtrate corresponded to 42.39130 cc urine, 20 cc to 4.239130 cc.

Twenty cc of the filtrate were treated each time by the Schöndorff method, consuming 30.1 cc of the acid present when 10 g phosphoric acid were used; the control determination, such as the determinations with 3 and 5 g phosphoric acid, gave inaccurate results due to experimental error and were not used for this reason. Twenty cc filtrate = 4.239130 cc urine contained 0.10535 g urea nitrogen.

In 5 cc urine there were 0,12419 g urea nitrogen = 0,2662849 g urea.

In 100 cc urine there were 2,48318 g urea nitrogen = 5,325739 g urea.

In 460 cc urine there were 11,436544 g urea nitrogen = 24,508514 g urea.

Twenty cc filtrate = 4,239130 cc were treated each time by the Kjeldahl method; on an average 30,4 cc of the acid present were used: 30,3 cc in the first experiment, 30,5 cc in the second experiment. Twenty cc filtrate treated according to Kjeldahl contained 0,106400 g nitrogen.

Filtrate II from 5 cc urine contained 0,125497 g nitrogen.
Filtrate II from 100 cc urine contained 2,509948 g nitrogen.
Filtrate II from 460 cc urine contained 11,545765 g nitrogen.

Of the nitrogen not precipitable by phosphotungstic acid, 99,013522% existed as urea, and 93,427819% of the total nitrogen existed as urea.

In 5 cc urine there are 0,13300 g nitrogen, in the residue + filtrate II there are 0,13372 g nitrogen, a difference of +0,00372 g. Of the total nitrogen of 0,133000 g in 5 cc urine, the residue nitrogen is 0,007875 g, the urea nitrogen is 0,1214159 g, the amino acid nitrogen is 0,001238 g.

Of the total nitrogen of 0,13300 g in 5 cc urine
the residue nitrogen comprises..... 5,921052%
the urea nitrogen comprises.....93,427819%
the amino acid nitrogen comprises..... 0,930827%.
The total is 100,279698% and the additional 0,279698% indicates the experimental error.

This experiment shows that an increase of amino acid nitrogen cannot be demonstrated; even the quantity of residue nitrogen fluctuated inside the known limits. The chief nitrogen-containing constituent of urine is urea with 93,43%. Urea secretion is proportional to total nitrogen secretion; indeed, 24,51 g urea and correspondingly 12,24 g total nitrogen were found. The amount of total nitrogen is multiplied by two (see page 198) to give the quantity of urea in whole numbers.

Case XXVII.

Thirty-fifth experiment on 29-30 December 1902.

Typhus abdominalis, in the third week of illness, temperature between 38,3-38,7° C. Urine quantity 575 cc, density 1,032, acid reaction.

Five cc of the urine were treated each time by the Kjeldahl method; one experiment was inaccurate and is omitted. In the other experiment, 34,3 cc of the 0,25 N sulphuric acid present were used for the neutralization of the ammonia evolved.

In 5 cc urine there were 0.12005 g nitrogen.
In 100 cc urine there were 2.40100 g nitrogen.
In 575 cc urine there were 13.80575 g nitrogen.

The total material precipitable from 5 cc urine by phosphotungstic hydrochloric acid was precipitated by 19.6 cc of this reagent. The residue from 5 cc urine was treated in the usual way and used 2 cc of the 0.25 N sulphuric acid present in both experiments.

The residue from 5 cc urine contained 0.00700 g nitrogen.
The residue from 100 cc urine contained 0.01400 g nitrogen.
The residue from 575 cc urine contained 0.80500 g nitrogen.

Fifty cc urine + 246 cc phosphotungstic hydrochloric acid produced a filtrate of 220 cc which increased in volume to 225 cc on the addition of lime; 225 cc filtrate correspond to 44.715447 cc urine, 20 cc to 3.974706 cc urine.

Twenty cc filtrate, treated by Schöndorff's[11] method, consumed these quantities of the acid present each time: using

10 g phosphoric acid, 24.8 cc of acid on the average were used:
24.8 cc in the first experiment, 24.8 cc in the second experiment;
5 g phosphoric acid, 24.65 cc of acid on the average were used:
24.6 cc in the first experiment, 24.7 cc in the second experiment;
3 g phosphoric acid, 24.3 cc of the acid on the average were used:
24.4 cc in the first experiment, 24.2 cc in the second experiment.

The number 24.8 was taken as the basis for further calculations.

Twenty cc of Filtrate II were treated each time by the Kjeldahl method; on the average 25.55 cc of the acid present were used: 25.5 cc in the first experiment, 25.6 cc in the second experiment.

According to the Kjeldahl analysis, 20 cc filtrate II = 3.974706 cc urine contained 0.089425 g nitrogen.

Filtrate II from 5 cc urine contained 0.112492 g nitrogen.
Filtrate II from 100 cc urine contained 2.249851 g nitrogen.
Filtrate II from 575 cc urine contained 12.939164 g nitrogen.

Of the nitrogen not precipitable by phosphotungstic acid, 97.06468% existed as urea; of the total nitrogen, 90.953769% existed as urea.

Five cc urine contained 0.12005 g nitrogen; in the residue + filtrate II there were 0.119492 g nitrogen, a difference of -0.000558 g. Of the nitrogen content of 0.12005 g in 5 cc urine, the residue nitrogen amounted to 0.007 g, the urea nitrogen amounted to 0.109190 g and the amino acid nitrogen amounted to 0.003302 g.

Of the total nitrogen of 0.12005 g in 5 cc urine,
the residue contained..... 5.830903%
the urea contained.....90.953769%
the amino acids contained..... 2.750520%.
The total is 99.535192% and the missing 0.464808% indicates the experimental error.

Case XXVIII.

Thirty-sixth experiment on 30-31 December 1902.

Typhus abdominalis, in the fourth week of illness, temperature between 37.2-39.1° C. Urine quantity 1108 cc, density 1.025, acid reaction.

Five cc urine were treated each time by the Kjeldahl method. An average of 23.55 cc of the 0.25 N sulphuric acid present was used: 23.5 cc in the first experiment, 23.6 cc in the second experiment.

In 5 cc urine there were 0.082425 g nitrogen.
In 100 cc urine there were 1.648500 g nitrogen.
In 1108 cc urine there were 18.265380 g nitrogen.

The total material precipitable from 5 cc urine by phosphotungstic hydrochloric acid was precipitated by 22.5 cc of this reagent. The residue from 5 cc urine was treated in the usual way, using 2.2 cc of the acid present, in both experiments.

The residue from 5 cc urine contained 0.00770 g nitrogen.
The residue from 100 cc urine contained 0.15400 g nitrogen.
The residue from 1108 cc urine contained 1.706320 g nitrogen.

Fifty cc urine + 225 cc phosphotungstic hydrochloric acid produced a filtrate of 250 cc which increased to a volume of 255 cc on the addition of lime; 255 cc filtrate correspond to 45.454545 cc urine, 20 cc filtrate to 3.565062 cc urine.

Twenty cc filtrate, treated by the Schöndorff method, consumed these quantities of the acid present each time: using

10 g phosphoric acid, on the average 14.3 cc acid were used:
14.3 cc in the first experiment, 14.3 in the second experiment;
5 g phosphoric acid, on the average 14.3 cc acid were used:
14.3 cc in the first experiment, 14.3 cc in the second experiment;
3 g phosphoric acid, on the average 14.3 cc acid were used:
14.3 cc in the first experiment, 14.3 cc in the second experiment.

Absolutely identical results were obtained in all experiments.

Twenty cc filtrate, corresponding to 3.565062 cc urine contained 0.050050 g urea nitrogen.

In 5 cc urine there were 0,070195 g urea nitrogen = 0,150427885 g urea.
In 100 cc urine there were 1,4039 g urea nitrogen = 3,0085577 g urea.
In 1108 cc urine there were 15,555241 g urea nitrogen = 33,334881463 g urea.

Twenty cc filtrate = 3,565062 cc urine were treated each time by the Kjeldahl method; 14,6 cc of the acid present were used up in both experiments. Twenty cc filtrate II = 3,565062 cc urine, treated by the Kjeldahl method, contained 0,05110 g nitrogen.

Filtrate II from 5 cc urine contained 0,071667 g nitrogen.
Filtrate II from 100 cc urine contained 1,433355 g nitrogen.
Filtrate II from 1108 cc urine contained 15,881575 g nitrogen.

Of the nitrogen not precipitable by the phosphotungstic hydrochloric acid, 97,940474% existed as urea, while 85,157415% of the total nitrogen existed as urea.

Five cc urine contained 0,082425 g nitrogen, the residue + filtrate II contained 0,079367 g nitrogen, a difference of -0,003058 g. Of the total nitrogen of 0,082425 g in 5 cc urine, the residue contained 0,0077 g nitrogen, the urea contained 0,070191 g nitrogen, and the amino acids contained 0,000976 g nitrogen.

Of the total nitrogen of 0,082425 g in 5 cc urine,
the residue contained..... 9,341838%
the urea contained.....85,157415%
the amino acids contained..... 1,184106%.
The sum is 97,940974% and the missing 4,31641% indicates the experimental error.

Case XXIX.

Thirty-seventh experiment on 4-5 January 1903.

Typhus abdominalis, in the second week of illness, temperature between 37,6-39,1° C. Urine quantity 530 cc (approximately), density 1,032, acid reaction, no albumen.

Five cc urine were treated each time by the Kjeldahl method. On the average, 36,85 cc of the normal sulphuric acid present were used: 36,7 cc in the first experiment, 37 cc in the second experiment.

In 5 cc urine there were 0,128975 g nitrogen.
In 100 cc urine there were 2,579500 g nitrogen.
In 530(?) cc urine there were 13,671350(?) g nitrogen.

The total material precipitable from 5 cc urine by phosphotungstic hydrochloric acid was precipitated by 24,5 cc of this reagent. The residue

from 5 cc urine treated in the usual way consumed an average of 2,2 cc of the acid present: 2 cc in the first experiment, 2,4 cc in the second experiment.

The residue from 5 cc urine contained 0,0077 g nitrogen.
The residue from 100 cc urine contained 0,1540 g nitrogen.
The residue from 530(?) cc urine contained 0,8162(?) g nitrogen.

Fifty cc urine + 245 cc phosphotungstic hydrochloric acid produced a filtrate of 270 cc which increased in volume to 275 cc on the addition of lime; 275 cc filtrate correspond to 45,762711 cc urine, 20 cc filtrate to 3,328197 cc urine.

Twenty cc filtrate, treated by the Schöndorff method, consumed these quantities of the acid present each time: using

10 g phosphoric acid, on the average 22,8 cc acid were used:
22,8 cc in the first experiment, 22,8 cc in the second experiment;
5 g phosphoric acid, on the average 22,8 cc acid were used:
22,8 cc in the first experiment, 22,8 cc in the second experiment;
3 g phosphoric acid, on an average, 22,35 cc acid were used:
22,4 cc in the first experiment, 22,3 cc in the second experiment.

Using 10 and 5 g of phosphoric acid gave identical results while using 3 g gave smaller numbers. The number 22,8 cc was taken as the basis for further calculations. Twenty cc filtrate corresponding to 3,328197 cc urine contained 0,081725 g urea nitrogen.

In 5 cc urine there were 0,119884 g urea nitrogen = 0,256911412 g urea.
In 100 cc urine there were 2,397694 g urea nitrogen = 5,138258242 g urea.
In 575(?) cc urine there were 12,707781(?) g urea nitrogen = 27,232774683(?) g urea.

Twenty cc filtrate II = 3,328197 cc urine were treated each time by the Kjeldahl method; an average of 23,35 cc of the 0,25 N sulphuric acid present were used: 23,4 cc in the first experiment, 23,3 cc in the second experiment. Twenty cc filtrate II = 3,328197 cc urine, investigated by the Kjeldahl method, contained 0,081725 g nitrogen.

Filtrate II from 5 cc urine contained 0,122776 g nitrogen.
Filtrate II from 100 cc urine contained 2,455533 g nitrogen.
Filtrate II from 575(?) cc urine contained 13,014328(?) g nitrogen.

Of the nitrogen not precipitable by the phosphotungstic hydrochloric acid, 97,644490% existed as urea; 92,95134% of the total nitrogen existed as urea.

Five cc urine contained 0,128975 g nitrogen; the residue + filtrate

II from 5 cc urine contained 0.130476 g nitrogen. The difference is +0.001511 g. Of the 0.128975 g nitrogen in 5 cc urine, the residue contained 0.0077 g nitrogen, the urea 0.119884 g nitrogen, the amino acids 0.002892 g nitrogen.

Of the total nitrogen of 0.128975 g in 5 cc urine
the residue contained..... 5.970149%
the urea contained.....92.951347%
the amino acids contained..... 2.242372%.
The total is 101.163868% and the additional 1.163868% indicates the experimental error.

Case XXX.

Thirty-eighth experiment on 4-5 January, 1903.

Typhus abdominalis in the second of illness. Temperature between 38-39.1° C.

Urine quantity 820 cc, density 1.030, acid, no albumen.

Five cc urine were treated by the Kjeldahl method each time; one experiment was inaccurate. In the second experiment 35.3 cc of the 0.25 N sulphuric acid present was used.

In 5 cc urine there were 0.12355 g nitrogen.
In 100 cc urine there were 2.47100 g nitrogen.
In 820 cc urine there were 20.26220 g nitrogen.

The total material precipitable by phosphotungstic hydrochloric acid was precipitated from 5 cc urine by 23.8 cc of this reagent. The residue from 5 cc urine, treated in the usual way, used an average of 2.1 cc of the sulphuric acid present: 2 cc in the first experiment, 2.2 cc in the second experiment.

The residue from 5 cc urine contained 0.00735 g nitrogen.
The residue from 100 cc urine contained 0.14700 g nitrogen.
The residue from 820 cc urine contained 1.20540 g nitrogen.

Fifty cc urine + 238 cc phosphotungstic hydrochloric acid produced a filtrate of 260 cc which increased in volume to 265 cc on the addition of lime; 265 cc filtrate correspond to 45.138888 cc urine, 20 cc filtrate to 3.406708 cc urine.

Twenty cc filtrate, treated by the Schöndorff method, consumed these quantities of the acid present each time: using

10 g phosphoric acid, 22.9 cc of acid were used on an average:
22.9 cc in the first experiment, 22.9 cc in the second experiment;

5 g phosphoric acid, 22.85 cc of acid were used on an average:
22.9 cc in the first experiment, 22.8 cc in the second experiment;
3 g phosphoric acid, 22.35 cc of acid were used on an average:
22.1 cc in the first experiment, 22.6 cc in the second experiment.

The number 22.9 cc was taken as a basis for further calculations; identical results were obtained with 10 and 5 g phosphoric acid and smaller values with 3 g.

Twenty cc filtrate = 3.40678 cc urine contained 0.08015 g urea nitrogen.

In 5 cc urine there were 0.117685 g urea nitrogen = 0.252091805 g urea.
In 100 cc urine there were 2.352711 g urea nitrogen = 5.041860 g urea.
In 820 cc urine there were 19.292231 g urea nitrogen = 41.342510 g urea.

Twenty cc filtrate = 3.406708 cc urine were treated each time by the Kjeldahl method. In both experiments 23.5 cc of the acid present were used. Twenty cc filtrate II = 3.406708 cc urine, treated by the Kjeldahl method, contained 0.08225 g nitrogen.

Filtrate II from 5 cc urine contained 0.120717 g nitrogen.
Filtrate II from 100 cc urine contained 2.414354 g nitrogen.
Filtrate II from 820 cc urine contained 19.797704 g nitrogen.

Of the nitrogen not precipitable by phosphotungstic hydrochloric acid, 97.446921% existed as urea; 95.212464% of the total nitrogen existed as urea.

Five cc urine contained 0.12355 g nitrogen, the residue + filtrate II contained 0.128067 g nitrogen. The difference is +0.004517 g. Of the 0.12355 g nitrogen in 5 cc urine, the residue contained 0.00735 g, the urea contained 0.115874 g, the amino acids contained 0.003082 g.

Of the total nitrogen of 0.12355 g in 5 cc urine
the residue contained..... 5.949008%
the urea contained.....95.212464%
the amino acids contained..... 2.494536%.
The total is 103.656008% and the additional 3.656008% indicates the experimental error.

These results were obtained from the investigation of these four cases of typhus abdominalis.

The amino acid nitrogen was distributed between 1.18-2.49% in the total nitrogen secretion. The absolute values for the secretion of amino acid nitrogen in the 24 hour quantity of urine of the sick fluctuated between 0.22-0.51 g, 0.3670 g on the average. This quantity corresponded to the average of 4.9023 g hippuric acid in the daily quantity of urine.

Therefore, a moderate increase of the amino acid nitrogen was also found here, while in the first series of observations ([Note]: see R. v. Jaksch, loc. cit., p. 61), the numbers for the amino acid nitrogen were too large due to the use of too little phosphoric acid in the Schöndorff determination of urea. In the case of typhus, this error is all the more perceptible because of the higher urea content of the urine. As Table VI shows, the 3 g of phosphoric acid in experiments 38 and 39 did not suffice to convert all the urea present into ammonium phosphate, whereas in all the other experiments, with the exception of those with diabetic urine (see Table I and VI), even this 3 g quantity sufficed.

As for the urea, it is distributed in the total nitrogen secretion on the order of 85.16-95.21%, an average of 91.07% from four experiments. The absolute quantity of urea in a 24 hour quantity of urine amounts to 26.91-41.34 g. It is proportional to the total nitrogen secretion which amounts to 12.6-19.29 g. Multiplying this by two gives the approximate quantity of urea in whole numbers.

The chief product of nitrogen conversion during typhus is urea. Of course, almost the highest values for the absolute quantity of amino acid nitrogen in the daily quantity of urine were obtained here, 0.51 g in one case. Actually, therefore, an increase of amino acid nitrogen takes place during typhus.

Finally, as for the residue nitrogen which we related to the presence of purine materials and ammonia in the urine, the values fluctuate between 5.83-9.34%, an average of 6.77%. Therefore, these values do not depart from the values obtained for other illnesses. Thus, these materials do not play an essential role in the metabolism of typhoid. The metabolism of the typhoid is analogous to that of every fever illness: it causes the indications of increased formation and secretion of urea besides a slight increase of amino acid nitrogen.

The observations discussed here are compiled in the following tables.

Table V

Number of experiments	Case	Diagnosis	24h Quantity of Urine in cc	Density of the Urine	Total Quantity of Nitrogen in:			Residue Nitrogen in:			Urea Nitrogen in:			Urea in:		Total Amount of Nitrogen not Precipitable in:			Total Amount of Amino Acid in:			The percent of Nitrogen not Precipitable by Phosphotungstic Acid which Existed as Urea	Percentage of Total Nitrogen Contributed by:			
					5 ccm	100 ccm	Daily Quantity	5 ccm	100 ccm	Daily Quantity	5 ccm	100 ccm	Daily Quantity	100 ccm	Daily Quantity	5 ccm	100 ccm	Daily Quantity	5 ccm	100 ccm	Daily Quantity		Residue Nitrogen	Nitrogen in Albumen	Urea Nitrogen	Amino Acid Nitrogen
IV. Diabetes mellitus																										
30	XXIV	Diabetes mellitus vergohren	–	1,020 vor der Gährung 1,013 nach der Gährung	0,0214	0,4270	–	0,0091	0,1820	–	0,0035	0,1930	–	0,3629	–	0,0007	0,1935	–	0,0012	0,0242	–	87,67	12,62	0	39,65	5,67
V. Lobular Pneumonia																										
31	XXV	Lobulare Pneumonie	–	1,022	0,0598	1,1970	–	0,0026	0,0525	–	0,0548	1,0963	–	2,2637	–	0,0552	1,1039	–	0,0024	0,0476	–	95,68	4,39	0,29	88,25	3,96
32	"	Derselbe vergohren	–	–	0,0578	1,1550	–	0,0037	0,0735	–	0,0532	1,0449	–	2,2393	–	0,0503	1,0059	–	0	0	0	100,00	6,36	0	90,47	0
VII. Erysipelas																										
34	XXVI	Erysipel	460	1,017	0,1330	2,6000	12,2360	0,0079	0,1575	0,7245	0,1346	2,4852	11,4365	3,3257	24,5085	0,1255	2,7099	11,5458	0,0012	0,0248	0,1139	99,01	5,92	0	93,43	0,93
VIII. Typhus abdominalis																										
35	XXVII	Typhus abdominalis	575	1,032	0,1201	2,4010	13,8055	0,0070	0,0140	0,8050	0,1131	2,2838	12,5569	4,6788	26,9094	0,1125	2,2499	12,9392	0,0033	0,0660	0,3797	97,06	5,83	0	90,95	2,75
36	XXVIII	Typhus abdominalis	1100	1,025	0,0824	1,6485	18,2654	0,0077	0,1540	1,7061	0,0747	1,4320	15,5552	3,0687	33,3349	0,0717	1,4334	15,8516	0,0010	0,0195	0,2163	97,31	9,34	0	85,16	1,14
37	XXIX	Typhus abdominalis	5340	1,032	0,1290	2,5795	13,6714	0,0077	0,1540	0,8162	0,1199	2,3977	12,7078	5,1383	27,2328	0,1228	2,4555	13,0148	0,0029	0,0578	0,3066	97,04	5,97	0	92,95	2,24
38	XXX	Typhus abdominalis	830	1,030	0,1236	2,4719	20,2692	0,0074	0,1470	1,2094	0,1162	2,3527	19,2922	5,0418	41,3425	0,1207	2,4144	19,7977	0,0031	0,0616	0,5054	90,45	5,95	0	95,21	2,49

Col. 1 Col. 2

Column 1
Fermented diabetes mellitus
Lobular pneumonia
The same, fermented
Erysipelas
Typhus abdominalis, etc.

Column 2
Before fermentation
After fermentation

Before I pass on to the concluding observations, I will first of all answer the question encountered in experiments 17-44 [See Note] of whether less than 10 g of phosphoric acid are enough, as in urine originating from kidney diseases, to convert the urea present into ammonium phosphate.

[Note]: I have also assimilated into the Table experiments 39-44 which were first discussed on page 234.

First I will give the Table on the results concerning this, obtained from the observations.

(See Table VI below.)

These observations show that 3 g of tribasic phosphoric acid are enough in urine from liver diseases, urine from anemia, etc., to convert the urea contained in 5 cc urine into ammonium phosphate using Schöndorff's[19] method.

Table VI.

The Amount of Normal Sulphuric Acid in Cubic Centimeters Used Each Time by 20 cc Filtrate

Using:

	Number of Experiments		I	II	Average		I	II	Average		I	II	Average
Erysipelas	34.	10 g Tribasic Phosphoric Acid	30.1	—	30.1		—	—	—		—	—	—
Typhus	35.	10 g - -	24.8	24.8	24.8	from 5 g	24.6	24.7	24.65	from 3 g	24.4	24.2	24.3
	36.	10 g - -	14.3	14.3	14.3	- 5 g	14.3	14.3	14.3	- 3 g	14.3	14.3	14.3
	37.	10 g - -	22.8	22.8	22.8	- 5 g	22.8	22.8	22.8	- 3 g	22.4	22.3	22.3
	38.	10 g - -	22.9	22.9	22.9	- 5 g	22.9	22.8	22.85	- 3 g	22.1	22.6	22.3

The urine of diabetics and each case in which the urine contained sugar behaved differently as I already explained on page 206. As the Table shows, 10, even 15 g were insufficient in this case, and 20 g of phosphoric acid had to be used. These results, however, are not reliable and it is recommended that the urine be fermented before carrying out the urea determination (see page 210). Five or even three grams of phosphoric acid were sufficient to convert all the urea into ammonium phosphate in urine which contained only small amounts of sugar (see experiments 31 and 32).

The urine of the typhus victim behaved somewhat differently from that of the former group. Here, 5 g phosphoric acid were not enough, as Table VI and experiments 35-38 show, to convert all the urea into ammonium phosphate if the content were high (case 35).

If we summarize the material on page 189 and in Table I and that discussed here, we find that in the larger number of cases, by far, 5 g, even 3 g of phosphoric acid sufficed to produce accurate values using the Schöndorff method of urea determination. In the future, 5 g phosphoric acid will suffice in carrying out the procedure. In the case of urine from feverish people, 10 g are recommended; urine from diabetics should be previously fermented, or a much larger quantity, 20 g, of crystalline tribasic phosphoric acid should be used.

Here I would like to meet the objection (see page 191) that the quantities of amino acids found correspond only to the experimental error. If we exclude experiment 30 on the grounds explained on page 216, this error in the 21 experiments fluctuates between

$$\left.\begin{matrix}-0.4\text{-}6.4\% \\ +0.28\text{-}3.7\%\end{matrix}\right) \text{ of the total nitrogen (see pages 190 and 191).}$$

For this reason, I am compiling here part of Table III and Table VII and refer you to the details on page 191. Note that I also assimilated into this Table experiments 39-44 which are still to be discussed.

Table VII.

Experiment	Twenty cc Filtrate Used by the acid present: in the Kjeldahl method cc	in the Schöndorff method cc	Difference in cc
17	11.5	11.25	0.35
18	7.8	7.7	0.1
19	9.5	9.3	0.2
20	12.65	12.15	0.5
21	9.7	9.45	0.25
27	8.2	7.9	0.3
29	12.05	11.6	0.45
30	3.2	2.8	0.4
31	12.75	12.2	0.55
32	11.6	12.05	negative
33	11.95	11.35	0.6
34	30.4	30.1	0.3
35	25.55	24.8	0.75
36	14.6	14.3	0.3
37	23.3	22.8	0.5
38	23.5	22.9	0.6
39	15.55	15.2	0.25

Table VII. (Continued)

Experiment	Twenty cc Filtrate Used in the Kjeldahl method cc by the acid present	in the Schondorff method cc by the acid present	Difference in cc
40	16.2	16.05	0.05
41	14.15	13.7	4.45
42	12.2	11.98	0.27
43	11.7	11.45	0.25
44	15.8	15.7	0.1

Only those cases have been included in the Table in which the differences presented have resulted from true behavior of urine. Those observations, for example those on unfermented diabetic urine, in which the results and therefore the differences were inaccurate, were not presented. From 22 experiments it is clear, since the difference always lies between 0.1-0.75, that besides the urea there were one or more materials also present which were not precipitated by phosphotungstic acid. Thus it is demonstrated that small quantities of amino acids or materials like them are found in the urine and can be detected by means of the selected preceding method.

There can still be doubt of whether this method can generally detect special amino acids. For this purpose I determined the distribution of nitrogen in a mild case of rheumatism and administered sodium benzoate.

From a clinical view, these studies have shown:

1. Of the nitrogen from the urine of the sick that was not precipitable by phosphotungstic acid, 95.85-98.36% existed as urea.

2. Of the nitrogen-containing materials precipitable by phosphotungstic acid, 5.16-8.51% existed as purine materials and ammonia compounds.

3. Of the total nitrogen in urine, 83.93-91.07% originated from urea.

4. Amino acid nitrogen accounted for 1.52-3.61% of the total nitrogen.

5. The amount of total nitrogen, multiplied by two, gives the amount of urea in albumen-free urine with sufficient accuracy for clinical investigations.

6. According tc the relations found in pathological urine and from the two experiments on normal humans (experiments 47 and 48), about 1.5-3% at the most of the total nitrogen should consist of amino acid nitrogen; this quantity can be increased by certain nourishment, for example the introduction of materials containing benzoic acid (see experiments 39-44).

7. In liver diseases, typhus abdominalis, diabetes mellitus, even in individual cases of Basedow illness, the elimination of amino acid nitrogen increased. This can amount to 0.64 g in the daily quantity of urine during diabetes, 0.50 g in typhus.